

Patent Claims

1 1. A method of microbial production of amino acids of
2 aspartate and/or glutamate families in which the pyruvate-
3 carboxylase activity is increased by genetic modification of the
4 enzyme and/or the pyruvate-carboxylase gene expression of the
5 corresponding amino-acid-producing micro organism.

1 2. The method of claim 1, characterized in that, by
2 mutation of the endogenous pyruvate-carboxylase gene an enzyme with
3 higher pyruvate-carboxylase activity is produced.

A
1 3. The method of claim 1 ~~or 2~~, characterized in that,
2 the gene expression of the pyruvate-carboxylase is increased by
3 increasing the gene copy number.

1 4. The method according to claim 3, characterized in
2 that, to increase the gene copy number the pyruvate-carboxylase
3 gene is incorporated in a gene construct.

1 5. The method according to claim 3, characterized in
2 that, the gene is incorporated in a gene construct which contains
3 regulatory gene sequences associated with the pyruvate-carboxylase
4 gene.

1 6. The method according to claim 4 ~~or~~ 5, characterized
2 in that, the corresponding amino-acid-producing microorganism is
3 transformed with the gene-containing gene construct.

1 7. The method according to claim 6, characterized in
2 that, a microorganism of the species *Corynebacterium* is transformed
3 with the gene containing the gene construct.

1 8. The method according to claim 6 ~~or~~ 7, characterized
2 in that, for the transformation a microorganism is used in which
3 the enzyme participating in the synthesis of the corresponding
4 amino acid is deregulated and/or wherein an enhanced export carrier
5 activity is shown for the corresponding amino acid.

1 9. The method according to claim 6 ~~to 8~~, characterized
2 in that, for the transformation a microorganism is used which has a
3 higher proportion of the central metabolism metabolites of the
4 corresponding amino acid participating in the synthesis.

1 10. The method according to claim 6 ~~to 9~~, characterized
2 in that, for the transformation a microorganism is used in which
3 biosynthesis paths competing with the corresponding amino acid
4 biosynthesis paths runs with reduced activity.

1 11. The method according to ~~one of the preceding~~ claims, ¹
2 characterized in that, the pyruvate-carboxylase gene is isolated
3 from a microorganism strain of the variety *Corynebacterium*.

1 12. The method according to ~~one of the preceding~~ claims, ¹
2 characterized in that, the gene expression is increased by
3 reinforcement of the transcription signal.

1 13. The method according to ~~one of the preceding~~ claims, ¹
2 characterized in that, the pyruvate-carboxylase gene has the tac-
3 promot r ahead of the pyruvate-carboxylase gene.

1 14. The method according to claim 13, characterized in
2 that, the tac-promoter is associated with regulatory sequences.

1 15. The method according to ~~one of the preceding claims~~¹,
2 characterized in that, the pyruvate-carboxylase gene is a gene with
3 the amino acid sequence given under SEQ ID No. 2 and its allele
4 variation coding nucleotide sequences.

1 16. The method according to claim 15, characterized in
2 that, with the pyruvate-carboxylase gene a gene with the nucleotide
3 sequence of nucleotide 165 to 3587 according to SEQ ID No. 1 or a
4 substantially identically-effective DNA sequence is used.

1 17. The method according to ~~one of the preceding claims~~¹,
2 for the production of lysine, threonine, homoserine, glutamate
3 and/or arginine.

1 18. A pyruvate-carboxylase gene coding for the amino
2 acid sequence given under SEQ ID No. 2 and /or a nucleotide
3 sequence coding for its allele variations.

1 19. The pyruvate-carboxylase gene according to claim 18
2 with the nucleotide sequence of nucleotides 165 to 3587 according
3 to SEQ ID No. 1 or a substantially identically-effective DNA
4 sequence.

1 20. The pyruvate-carboxylase gene according to claim 18
2 ~~or 19~~ with a preceding promoter of the nucleotide sequence from
3 nucleotide 20 to 109 according to SEQ ID No. 1 or a substantially-
4 identically-effective DNA sequence.

5 21. The pyruvate-carboxylate gene according to claim 18
6 ~~or 19~~, with preceding tac-promoter.

7 22. The pyruvate-carboxylase gene according to claim 21
8 with the regulatory sequence associated with the promoter.

1 23. The pyruvate-carboxylase gene according to ~~one of~~
2 ~~claims 18 to 20~~ with these regulatory gene sequences associated
3 therewith.

1 24. A gene structure containing a pyruvate-carboxylase
2 gene according to ~~one of claims 18 to 23~~.

3 25. A vector containing a pyruvate-carboxylase gene
4 ~~according to one of claims 18 to 23~~ or a gene structure according
5 to claim ¹⁸~~24~~.

1 26. Transformed cells containing in replicatable form a
2 pyruvate-carboxylase gene ~~according to one of claims 18 to 23~~ or a
3 gene structure according to claim ¹⁸~~24~~.

1 27. Transformed cells ~~according to claim 26~~ containing a
2 vector according to claim 25.

1 28. Transformed cells according to claim 26 ~~or 27~~,
2 characterized in that, they belong to the variety *Corynebacterium*.

1 29. Transformed cells according to ~~one of claims 26 to~~
2 ~~28~~, characterized in that, enzymes which participate in the
3 synthesis of the corresponding amino acid and/or enzyme which
4 participate in the export of the corresponding amino acid are
5 deregulated.

6 30. Transformed cells according to ~~one of claims 26 to~~
7 ~~29~~, characterized in that, they contain an increased proportion of
8 the central metabolism metabolites participating in the synthesis
9 of the corresponding amino acid.

1 31. Transformed cells according to ~~one of claims 26 to~~
2 30, characterized in that, they contain a reduced proportion of the
3 central metabolism metabolites which do not participate in the
4 synthesis of the corresponding amino acid.

1 32. The use of a pyruvate-carboxylase gene for
2 increasing the production of amino acids of the aspartate and/or
3 glutamate families by microorganisms.

1 33. The use according to claim 32, characterized in
2 that, a mutated pyruvate-carboxylase gene which codes for an enzyme
3 with increase pyruvate-carboxylase activity is used.

1 34. The use according to claim 32 ~~or 33~~, characterized
2 in that, the microorganism producing the corresponding amino acid

3 is transformed with a gene construct that contains a pyruvate-
4 carboxylase gene.

1 35. The use according to claim 34, characterized in
2 that, the gene construct additionally contains regulatory gene
3 sequences.

1 36. The use according to ~~one of claims 32 or 35,~~
2 characterized in that, a pyruvate-carboxylase gene from
3 *Corynebacterium* is used.

1 37. The use according to ~~one of claims 32 or 36,~~
2 characterized in that, *Corynebacterium* is used as the amino acid-
3 producing microorganism.

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